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(54) Title: METHODS FOR TREATING PLANTS AND ENHANCING PLANT GROWTH WITH CONJUGATED INDOLES AND FORMULATIONS FOR SAME

(57) Abstract: Methods and formulations for treating plants and enhancing plant growth, whereby one or more compounds, selected from a group consisting of conjugated indoles, salts and derivatives of conjugated indoles, and combinations thereof, are applied to the plant in an effective amount.

# Methods for Treating Plants and Enhancing Plant Growth With Conjugated Indoles and Formulations for Same

This invention relates to methods and formulations for treating plants, and more specifically to methods for treating plants with formulations comprising conjugated indoles.

The growth of plants is dependent on the synthesis of polysaccharides, especially, cellulose in cell walls, but the primers for chain initiation and the substrates for chain extension have not been previously defined. Although substrates such as UDP-Glc have been proposed and glucosyltransferases have been identified, the biosynthetic pathway has not been previously defined. Similarly, general metabolic pathways for plant growth regulators (PGRs) have also not been completely defined, although numerous PGR compounds have been identified over the past six decades.

Recently, it was reported that dodecylmaltoside and related compounds can serve as substrates for glycogen synthase. Glycogen, the reserve carbohydrate in animals, is chemically distinct from plant reserves and glycogen synthase has only rarely been described in lower

plants, although biochemical pathways for plant carbohydrates may be related. Further, in U.S. Patent Number 5,958,104, Nonomura et al. describe methods for applying linear $C_1$-$C_7$ alkyl glucosides to plants to enhance plant growth and yield. Nonomura et al. also disclose that PGRs could be applied along with the linear $C_1$-$C_7$ alkyl glucosides. On the other hand, conjugated plant growth regulators (CPGR5) have continued to be defined as the inactivated form of PGRs and, as such, no activity is expected by treating plants with CPGRs. For example, the conjugated auxin, indoxyl-beta-glucoside and the conjugated cytokinin BAP-9-glucoside were specifically noted, by Jasik et al., to have no marked effect on root development. Jasik et al., "Characterisation of morphology and root formation in the model wood perennial shrub *Solanum aviculare* Forst. expressing rolABC genes of *Agrobacterium rhizogenes*, "Plant Science, Vol. 124, No. 1 1997, pp. 57-68.

It is a primary object of the invention to provide methods and formulations for treating plants and enhancing plant growth by applying a formulation comprising one or more conjugated indole compounds to the plants.

It is a further object of the invention to provide methods and formulations for treating plants and enhancing plant growth by applying a formulation comprising one or more compounds selected from a group consisting of conjugated indoles, such as indoxyl glycoside; salts and derivatives of said conjugated indoles and combinations thereof, to the plants.

It is a further object of the invention to provide methods and formulations for treating plants and enhancing plant growth by applying a formulation of one or more synthetic components of conjugated indoles, such as indoxyl glycoside, to the plants.

It is a further object of the invention to provide methods and formulations for treating plants and enhancing plant growth by applying a formulation of one or more conjugated indoles and one or more phytocatalysts to the plants.

It is a further object of the invention to provide methods and formulations for treating plants and enhancing plant growth by applying one or more activated, conjugated indoles to the plants.

It is a further object of the invention to provides methods and formulations for treating plants and enhancing plant growth by applying one or more compounds selected from a group consisting of cyclic alkyl glycosides; salts and derivatives of cyclic alkyl glycosides; cyclic acyl glycosides: salts and derivatives of cyclic acyl glycosides; and combinations thereof, to the plants.

As noted, the prior art has consistently viewed CPGRS as the inactivated form of PGRS and therefore, incapable of eliciting any plant growth activity by exogeneously applying or making CPGRs available to the plant. However, contrary to prior teachings, the methods and formulations of the invention apply CPGRs to the plants to act as artificial substrates for carbohydrate synthase having recognized that most CPGRs are cyclic alkyl glycosides and that, as storage products, CPGRs are found in plants at over twenty times the concentration of their respective PGRs. For example, if, as previously thought, CPGRs were merely inactive storage products, then exogenous or endogenous release of PGRS would yield consistent growth enhancement, yet this is not the case. See e.g., M. Faiss, et al, "Chemically induced-expression of the rolC-encoded β-glucosidase in transgenic tobacco plants and analysis of cytokinin metabolism: rolC does not hydrolyze endogenous cytokinin glucosides in plants" The Plant Journal, 1O(1):33-46 (1996).

Furthermore, in crops, such as rice, yields have proven to be carbon sink limited. Cellulose is the largest sink in any plant and the application of cylic alkyl glycosides to allocate carbon into the largest sink may open crops to the proportionate enhancement of yield potential. See e.g., J.C. Waterlow et al., "Applications of science to increase yield', Chapter III, Feeding a World Population of More Than Eight Billion People (Oxford Univ. Press, 1998).

The methods and formulations of the invention were developed on the basis that conjugated plant growth regulators (CPGRs) are active contributors to polysaccharides that make up the cell wall of plants. Specificity resulting in carbon partitioning in plants is determined by the class of PGR that is conjugated to the glycoside. The invention, therefore, describes methods for promoting plant growth based on novel treatment regimes with CPGRs, and more specifically, with one or more compounds comprising indoxyl glycosides. When CPGRS are made available to plants in concentrations that are 30 to 300 times the cellular concentration of PGRS, the CPGRs become activated and deposit glucan units to prime and extend polymer chains. High quantities of CPGRs are required for activation because the CPGRs function both as primers and substrates for cellulose synthase. After deposition of the conjugate, the indole moiety is cut away and is further metabolized to transport glucans resulting from photosynthesis.

A preferred method of the invention, for treating plants and for enhancing plant growth, comprises the step of, applying an effective amount of one or more compounds selected from a group consisting of conjugated indoles; salts and derivatives of said conjugated indoles and combinations thereof, to said plant; wherein said effective amount is preferably between 1 ppm to 2,500 ppm.

One or more of said compounds may comprise indoxyl glycoside, wherein an effective amount preferably comprises indoxyl glycoside in an amount between about 1 mM to 6 mM concentration. In addition or alternatively, one or more of said compounds may comprise indoxyl glucoside; indoxyl glucuronide; indoxyl mannoside; isatin; isatan; isatoxime; indirubin; indole carboxylate; indoxyl acylglycosides; indoxyl $(acetyl)_n$glycosides where n=1-5, such as indoxyl $(acetyl)_5$glycoside; and/or isomers and salts thereof.

The method may further comprise the step of applying one or more phytocatalysts, wherein one or more of said phytocatalysts preferably comprises one or more nutrients selected from a group consisting of iron, manganese and nitrogen; wherein the nitrogen nutrient preferably comprises ammoniacal nitrogen. One or more of the phytocatalysts preferably comprises ammonium at 100 millimolar concentration $\mp$ 20 percent; manganese at 30 parts per million concentration $\mp$ 20 percent; and iron at 15 parts per million concentration $\mp$ 50 percent.

Another preferred method of the invention for treating plants and enhancing plant growth, comprises the step of, applying one or more compounds selected from a group consisting of cyclic alkyl glycosides; salts and derivatives of the cyclic alkyl glycosides; cyclic acyl glycosides; salts and derivatives of the cyclic acyl glycosides; and combinations thereof, to the plants; wherein one or more of the compounds preferably comprises one or more indoxyl glycosides; salts and derivatives of indoxyl glycosides and combinations thereof.

A preferred formulation of the invention for treating plants and for enhancing plant growth, comprises, one or more compounds selected from a group consisting of indoxyl glycosides, salts and derivatives of said indoxyl glycosides and combinations thereof; wherein

one or more of said compounds may be selected from a group consisting of, indoxyl glucoside, indoxyl glucuronide, indoxyl mannoside, isatin, isatan, isatoxime, indirubin, indole carboxylate, indoxyl $(acyl)_n$glycoside, and isomers and salts thereof. The indoxyl $(acyl)_n$glycoside may comprise indoxyl $(acetyl)_n$glycoside wherein n=1-5, such as indoxyl $(acetyl)_5$glucoside. The formulation also preferably comprises one or more more surfactants and/or one or more phytocatalysts comprising one or more nutrients selected from a group consisting of iron, manganese and nitrogen; wherein the nitrogen nutrient preferably comprises ammoniacal nitrogen. Similarly, one or more of the phytocatalysts preferably comprises ammonium at 100 millimolar concentration $\mp$ 20 percent; manganese at 30 parts per million concentration $\mp$ 20 percent; and iron at 15 parts per million concentration $\mp$ 50 percent.

Another preferred formulation of the invention for treating plants and for enhancing plant growth, comprises: one or more indoxyl glycosides in an amount between about 1 mM to 6 mM concentration; and one or more nutrients selected from a group consisting of iron, manganese and nitrogen; and preferably further comprises one or more surfactants.

Yet another preferred formulation of the invention for treating plants and enhancing plant growth, comprises, one or more compounds selected from a group consisting of cyclic alkyl glycosides; salts and derivatives of the cyclic alkyl glycosides; cyclic acyl glycosides; salts and derivatives of the cyclic acyl glycosides; and combinations thereof; wherein one or more of the compounds preferably comprises one or more indoxyl glycosides; salts and derivatives of the indoxyl glycosides and combinations thereof.

As noted, the methods and formulations of the invention are designed to treat plants and to enhance plant growth. Treatment and plant growth enhancement are generally achieved by formulating one or more conjugated indoles with one or more phytocatalysts and with or without one or more PGRs and applying the formulation in a dry or liquid form directly to the plants and/or the plant soil. Specifically, the formulations provide the plant with indigo and/or synthetic components of indigo, to enhance cellulose synthesis, wherein the components may include, but not limited to, indole carboxylate, indoxyl $(acetyl)_5$glycoside, isatin, isatan, isatoxime. indirubin and nitrobenzaldehydeindogenide, which stimulates plant growth by catalyzing endogenous biosynthesis of indoxyl glycoside and other conjugated auxins.

Unless otherwise defined, all technical and scientific terms employed herein have their conventional meaning in the art. As used herein, the following terms have the meanings ascribed to them.

"Enhance(s) growth" or "enhancing growth" refers to promoting, increasing or improving the rate of growth of the plant or increasing or promoting an increase in the size of the plant. Without wishing to be bound by any particular theory regarding the mechanism by which the compositions of the present invention enhance the growth of a plant, it is believed that when cellulose synthetase enzymes are induced exogenously by conjugated indoles in the presence of phytocatalysts, they are enhanced beyond the natural content of a plant and, thereby, lead to the enhanced growth of the plant. Exogenous enhancement of conjugated indoles increases the capacity of an organism to transport and glycosylate cellulose.

"Plant" refers to any life form which synthesizes cellulose including, but not necessarily limited to: microbials including prokaryotes, eukaryotes, bacteria, algae, lichens and fungi; cryptophytes; angiosperms; and gymnosperms. The methods and formulations of the inventions are advantageous for many applications including, but not limited to, agricultural, horticultural, maricultural, floricultural and silvicultural applications.

"Surfactant" refers to surface-active agents, i.e., which modify the nature of surfaces, often by reducing the surface tension of water. They act as wetting agents, spreaders, dispersants, or penetrants. Typical classes include cationic, anionic (e.g., alkylsulfates), nonionic (e.g., polyethylene oxides) and ampholytic. Soaps, alcohols, block copolymers and polysiloxanes are other examples.

"Aqueous", with reference to solutions or solvents, refers to solutions or solvent systems which consist primarily of water, normally greater than 50 weight percent water, and can be essentially pure water in certain circumstances. For example, an aqueous solution or solvent can be distilled water, tap water, irrigation water, well water or the like. However, an aqueous solution or solvent can include water having substances such as pH buffers, pH adjusters. organic and inorganic salts, alcohols (e.g., ethanol), sugars, amino acids, or surfactants incorporated therein. The aqueous solution or solvent may also be a mixture of water and minor amounts of one or more cosolvents, including agronomically suitable organic cosolvents, which are miscible therewith, or may form an emulsion therewith. Agronomically suitable organic solvents include, for example, acetone, methanol, limonene, paraffin oils, silanes, esters, ethers, and emulsifiers.

"Percent" or "percent" is percent by weight unless otherwise indicated.

"Ppm" refers to parts per million by weight.

"M" refers to molar concentration, "mM" refers to millimolar concentration, and "μM" refers to micromolar concentration.

"Auxin" is a plant hormone that is currently classified as a PGR which is physiologically active at 0. 1 to 1 ppm concentrations as a cell elongation factor or rooting stimulant found in plants.

"Cytokinin" refers to a PGR, generally with an adenine nucleus, that is physiologically active at very low concentration as a cell division factor found in plants and yeast.

"GA" refers to gibberellins, a class of over 60 PGRs that are diterpenoid acids based on the gibberellane skeleton containing the gibbane nucleus.

"PGR" refers to a plant growth regulator.

"PGRs" is the plural of PGR.

"PGR-glycoside" refers to glycoside-conjugated plant growth regulator compounds listed herein and those known in the field. Prior to this invention, PGR-glycosides were conventionally regarded as the inactivated form of the PGR. The glycoside component includes pentopyranosides, hexopyranosides, and so forth. Although cytokinin-glycosides, auxinglycosides, and gibberellin-glycosides have been identified in tissues, none have yet been previously applied to plants to enhance crop yields.

"CPGR" refers to a conjugated plant growth regulator.

"Alkyl glycoside" refers to glycoside-conjugated alkyls, including all isomers, that are saturated or unsaturated; and may be cyclic, heterocyclic, aromatic, substituted aromatic,

or heteroaromatic; salts and derivatives thereof; and any combination thereof.

"Cyclic alkyl glycoside" refers to cyclic, glycoside-conjugated alkyls, including all isomers, that are saturated or unsaturated; and may be heterocyclic, aromatic, substituted aromatic, or heteroaromatic; salts and derivatives thereof; and any combination thereof.

"Cyclic acyl glycoside" refers to cyclic, glycoside-conjugated acyls, including all isomers, that are saturated or unsaturated; and may be heterocyclic, aromatic, substituted aromatic, or heteroaromatic; salts and derivatives thereof; and any combination thereof.

"Conjugated indole" refers to glycoside-conjugated indoles, including all isomers, salts and derivatives thereof; and any combination thereof.

The resulting mixture of the method of the invention may be applied to all parts of the plant including the leaves, shoots, roots, stems, flowers and fruits, depending on the nature of the formulation utilized.

The formulations employed in the methods of the present invention may be applied to the plants using conventional application techniques. Plants nearing or at maturity may be treated at any time before and during seed development. Fruit bearing plants may be treated before or after the onset of bud or fruit formation. Improved growth occurs as a result of the exogenous application of high concentrations of soluble manganese with one or more conjugated indoles and other appropriate nutrients and additives such as ammoniacal nitrogen and soluble iron.

The conjugated plant growth regulators which may be activated using the formulations of the present invention include, but are not necessarily limited to:

PGR-glycosides including, but not limited to,

Indoxyl glycoside (otherwise known as indican)

- Indoxyl glucoside
- Indoxyl galactoside
- Indoxyl erythroside
- Indoxyl threoside
- Indoxyl riboside
- Indoxyl arabinoside
- Indoxyl xyloside
- Indoxyl lyxoside
- Indoxyl alloside
- Indoxyl altroside
- Indoxyl mannoside

Indoxyl guloside

Indoxyl idoside

Indoxyl taloside

Indoxyl erythruloside

Indoxyl ribuloside

Indoxyl xyluloside

Indoxyl psicoside

Indoxyl fructoside

Indoxyl sorboside

Indoxyl tagatoside:

Indolyl $(acetyl)_n$glycoside, where n= 1-5

Indolyl $(acetyl)_n$glucoside

Indolyl $(acetyl)_n$galactoside

Indolyl $(acetyl)_n$erythroside

Indolyl $(acetyl)_n$threoside

Indolyl $(acetyl)_n$riboside

Indolyl $(acetyl)_n$arabinoside

Indolyl $(acetyl)_n$xyloside

Indolyl $(acetyl)_n$lyxoside

Indolyl $(acetyl)_n$alloside

Indolyl $(acetyl)_n$altroside

Indolyl $(acetyl)_n$mannoside

Indolyl $(acetyl)_n$guloside

Indolyl $(acetyl)_n$idoside

Indolyl $(acetyl)_n$taloside

Indolyl $(acetyl)_n$erythruloside

Indolyl $(acetyl)_n$ribuloside

Indolyl $(acetyl)_n$xyluloside

Indolyl $(acetyl)_n$psicoside

Indolyl $(acetyl)_n$fructoside

Indolyl $(acetyl)_n$sorboside

Indolyl $(acetyl)_n$tagatoside;

Any other indole groups conjugated with:

Aldoses, such as,

glyceraldehyde
erythrose
threose
ribose
arabinose
xylose
lyxose
allose
altrose
glucose
mannose
gulose
idose
galactose
talose

Ketoses, such as,

dihydroxyacetone
erythrulose
ribulose
xylulose
psicose
fructose
sorbose
tagatose

Furanose
Pyranose
Glucopyranose
Fructofuranose
Fructopyranose
Xylopyranose

and their derivatives, e.g., glucuronides, glucosamines; and

Any conjugated indole isomer, metabolite, salt, hydrate, ester, amine, surfactant-linked derivative and other suitable biologically or chemically equivalent derivative and combination thereof.

Glycosylators (glycosylation substrates) useful in the formulations and methods of the invention include, but are not necessarily limited to:

Alcohol

Aldehyde

Carbonate

Carbon dioxide

Formate

Formamide

Ketone

Pentosan

Sugar

Their derivatives and the like.

The phytocatalysts of the formulations and methods of the invention preferably comprise manganese, iron and ammoniacal nitrogen sources in forms which are available to plants, which include, but are not necessarily limited to the following:

<u>Ammoniacal nitrogen</u>

Ammonium salts including, but not limited to:

- Ammonium sulfate
- Ammonium nitrate
- Ammonium formate
- Ammonium hydroxide
- Ammonium chloride

Urea

Formaldehyde urea

Amino Acid

Protein

Peptide

Manure

Guano

and any other acceptable fertilizer.

<u>Manganese</u>

Manganese salt

Manganese chelate

- Mn-EDTA

Mn-HEDTA

Mn-EDDHA

<u>Iron</u>

Ferric salt

Ferrous salt

Ferrous chelate

Ferric chelate

Fe-EDTA

Fe-HEDTA

Fe-EDDHA.

The formulations and methods of the present invention may be applied to virtually any variety of living organisms which synthesize cellulose. Such organisms, as noted above, include innumerable agricultural plants, such as those listed by G.M. Markle, J.J. Baron and BA. Schneider, <u>Food and Feed Crops of the United States</u>, (Meister Publishing 1998); and by Mark Griffiths, <u>Index of Garden Plants</u>, (Timber Press 1994). Further, plants which may benefit according to the present invention include but are not limited to all plants that have been genetically modified including hybridized, chimeric, transgenic, cross-bred, mutated, and plants which include recombinant DNA or RNA or have had their DNA or RNA otherwise modified or introduced. These lists are intended to be exemplary and are not intended to be exclusive. Other plants which may benefit by application of the compositions and methods of the present invention will be readily determined by those skilled in the art.

The methods and compositions of the present invention may be used to enhance growth in juvenile and mature plants, as well as cuttings, stolons, bulbs, rhizomes, micropropagative tissue, calli, protocorms, and seeds. Generally, however, it is desirable that, for foliar applications, the plants include at least the sprouted cotyledon (i.e., the "seed leaves') and preferably at least two additional expanded true leaves. Sprouted cotyledon and two expanded leaves are also preferred for root applications because the leaf development is, to some extent, indicative of root development. In general, roots may be treated because many plant growth regulators are transported up to shoots from roots.

**<u>Methods and Compositions</u>**

The present invention provides methods for treating plants, for increasing the amount of one or more conjugated indoles in a plant, and for enhancing the growth of the plant. These methods typically involve the application of an indole component, the application of a glycoside component, and the application of a phytocatalyst component to

the plant. In the event that a indole glycoside is available, these methods preferably involve the application of the indole glycoside and the application of phytocatalyst component to the plant.

### A. Indole glycoside

Conjugated indoles, such as indoxyl glycoside, are compounds which generally occur naturally in plants. According to the methods, compositions, and systems of the present invention, crop yields can be enhanced effectively and consistently by providing the glycosylator and phytocatalyst to an indole component. For high potency response, indole glycosides may be applied to the plant in place of the two components, indole and glycosylator, in accordance with the methods and compositions of this invention. Indoxyl glycoside utilized in the methods and formulations of the invention is commercially available and may also be synthesized according to known methods such as the method for synthesizing indoxyl-β-D-glucoside disclosed taught by A. Robertson, J. Chem. Soc., 1937.

Any number of indole glycoside compounds, such as indoxyl glycoside, may be used in the methods and formulations of the invention, including, but not limited to, those specifically listed above, as well as, metabolites, and all salts, hydrates, esters, amines, surfactant-linked derivatives, and other-biologically or chemically equivalent derivatives and combinations thereof. Generally, in the methods and formulations of the invention, the ratio of dry weight indigo applied to dry weight plant is approximately 5000:1.

### B. Glycosylator

Preferred glycosylator compounds are available organic or inorganic carbon compounds which can be metabolized by the plants to indoxyl-glycosides. Glycosylators must be applied to the plant in combination with phytocatalyst and may be further enhanced by formulation with indoxyl. Examples of suitable glycosylators include but are not limited to organic and inorganic carbon compounds. General examples of organic compounds include alcohol, aldehyde, ketone, organic acid, sugar, pentosan, alkyl glycoside, listed hereinabove as well as, metabolites, and all salts, hydrates, esters, amines, surfactant-linked derivatives, and other biologically or chemically equivalent derivatives thereof and combinations thereof. Specific examples of organic compounds include methanol, ethanol. propanol, acetone, formate, formamide formimide, citrate, lactate. salicylate, urea-formaldehyde, methyl glucoside, ethyl glucoside, propyl glucoside, fructose, ribose, xylose, methyl xyloside, corn syrup, molasses, maltose, PelRig® and Triazone®, listed hereinabove as well as, metabolites, and all salts, hydrates, esters, amines, surfactant-linked derivatives, and other biologically or chemically equivalent derivatives thereof and combinations

thereof. Specific examples of inorganic carbon include carbon dioxide; carbonate; and bicarbonate, such as ammonium bicarbonate, potassium bicarbonate, and sodium bicarbonate; as well as, metabolites, and all salts, hydrates, esters, amines, surfactant-linked derivatives, and other biologically or chemically equivalent derivatives thereof and combinations thereof.

**C. Phytocatalyst**

The phytocatalyst of the invention comprises soluble ammoniacal nitrogen, manganese and iron. The phytocatalyst is preferred in formulations of glycosylators and indoxyl-glycosides. Specific examples of ammoniacal nitrogen compounds include, but are not limited to, ammonium salts such as ammonium formate, ammonium citrate, ammonium lactate, ammonium salicylate, ammonium nitrate, ammonium sulfate and the like; urea-compounds such as urea, urea-formaldehyde; Triazone® and other Schiff-base compounds; quaternary amines; amino acids such as glycine, glutamine, tyrosine; protein; peptide; manure; fish meal; other sewage-based fertilizers; night soil; guano; nucleotide; purine; pyrimidine; amide; and imide; as well as, metabolites, and all salts, hydrates, esters, amines, surfactant-linked derivatives, and other biologically or chemically equivalent derivatives thereof and combinations thereof.

Specific examples of soluble manganese include manganese chelate such as Mn-EDTA, Mn-HEDTA. Mn-ascorbate, and the like; and manganese salts such as manganese chloride, and the like; listed hereinabove as well as, metabolites, and all salts, hydrates, esters, amines, surfactant-linked derivatives, and other biologically or chemically equivalent derivatives thereof and combinations thereof.

Specific examples of soluble iron include iron chelate such as Fe-EDTA, Fe-HEDTA, Fe-citrate, and the like; and ferric salts such as ferric chloride, ferric ammonium sulfate and the like; and ferrous salts such as ferrous sulfate and the like; listed hereinabove as well as, metabolites, and all salts, hydrates, esters, amines, surfactant-linked derivatives, and other biologically or chemically equivalent derivatives thereof and combinations thereof.

The following is an example of the preferred phytocatalyst formulation for use with the methods and formulations of the invention.

**PHYTOCATALYST EXAMPLE** - Foliar

| Component | Preferred concentration |
|---|---|
| $(NH_4)$ | 100mM ∓ 20 percent |
| Mn | 30ppm ∓ 20 percent |
| Fe | 15ppm ∓ 50 percent |

The above phytocatalyst formulation is calibrated to 20 gallons per acre for a standard foliar application rate per volume.

**D. Application**

Typically, the indole component and the glycosylator component are co-applied with the phytocatalyst component to achieve beneficial results in the methods for treating plants, enhancing growth, and increasing cellulose biosynthesis in photosynthetic plants. The methods of the present invention include the simultaneous application of the indole with the glycosylator and the phytocatalyst from separate sources; or the separate application of the indoxyl and glycosylator and phytocatalyst, wherein, the phytocatalyst is applied first followed by the application of the glycosylator and then followed by the indole; or by order of the separate application of the indole and the phytocatalyst wherein the phytocatalyst is applied first followed by the application of the indole and then the glycosylator. The phytocatalyst and the glycosylator and the indole may be applied separately, or formulated together and then applied, to the roots and/or the shoots in any combination or sequence such as those described above. The reverse orders may be applicable, but are not preferred. When the phytocatalyst and glycosylator and indole are separately applied, they are preferably applied at or near the same time, and generally one is applied within a four hour period of the other, preferably within an hour period, more preferably within a half hour period and most preferably within a quarter hour period. In the preferred method, the phytocatalyst plus glycosylator plus indole are formulated into a single composition and thereby simultaneously applied to the plant.

Although the components may be applied in a solid form, it is often advantageous to provide the formulation in liquid form, such as by solubilizing the components in an aqueous or agronomically suitable organic solvent or carrier to produce aqueous or organic solutions for application to the plant. The amount of indole, glycosylator, indole-glycoside, and phytocatalyst which is solubilized in the carrier will depend upon the particular compounds selected and the method of application. For example, indoxyl glycoside may be solubilized in the carrier by adding the indoxyl glycoside to the carrier and allowing it to

dissolve. In some instances, the application of stirring, agitation, or even heat may facilitate the dissolution of the indican in a carrier blend such as 80 percent ethanol. Typically, the indoxyl glycoside is applied as an aqueous solution having an indoxyl glycoside concentration in the range between 1 ppm and 2500 ppm by weight of the composition inclusive, preferably between 10 ppm and 1000 ppm, inclusive, for application to open field crops at a rate of 20 gallons per acre.

Typically, the co-application of a conjugated indole with a phytocatalyst is undertaken to achieve beneficial results in the methods for treating plants. For example, a conjugated indole, such as indoxyl glycoside, may be formulated with the phytocatalyst formulation, such as ammoniacal nitrogen, soluble Mn and soluble Fe.

The phytocatalyst is preferably applied first followed by the separate application of the indoxyl glycoside, however, the indoxyl glycoside may be applied first followed by the application of the phytocatalyst. The phytocatalyst and indoxyl glycoside may be separately applied, or formulated together and then applied, to the roots and/or the shoots in any of the above noted combinations or sequences. Other orders may be utilized, but are not preferred. When the phytocatalyst and indoxyl glycoside are separately applied, they are typically applied at or near the same time, and, generally, one is applied within a four hour period of the other, preferably within an hour period, more preferably within a half hour period and most preferably within a quarter hour period. In the preferred application method, the phytocatalyst plus indoxyl glycoside are formulated into a single composition and thereby simultaneously applied to the plant.

While the compositions of the present invention may consist essentially of the aqueous solutions of the indole, glycosylator, indole glycoside, and phytocatalyst, oil soluble compounds may be formulated in agronomically suitable organic solvents. For example, indican and the phytocatalyst may be formulated as isopropanol concentrates with paraffin oil as the spreader for application in appropriate crop emulsions, hydrosols or organic films.

The compositions of the present invention may also include any of a wide variety of agronomically suitable additives, adjuvants, or other ingredients and components which improve or at least do not hinder the beneficial effects of the compositions of the present invention (hereinafter "additives"). Generally accepted additives for agricultural application are periodically listed by the United States Environmental Protection Agency. For example, foliar compositions may contain a surfactant and a spreader present in an amount sufficient to promote wetting, emulsification, even distribution and penetration of the active

substances. Spreaders are typically organic-alkanes, alkenes or polydimethylsiloxanes which provide a sheeting action of the treatment across the phylloplane. Suitable spreaders include paraffin oils and polyalkyleneoxide polydimethylsiloxanes. Suitable surfactants include anionic, cationic, nonionic, and zwitterionic detergents, amine ethoxylates, alkyl phenol ethoxylates, phosphate esters, PEG, polymerics, polyoxyethylene fatty acid esters, polyoxyethylene fatty diglycerides, sorbitan fatty acid esters, alcohol ethoxylates, sorbitan fatty acid ester ethoxylates, ethoxylated alkylamines, quaternary amines, sorbitan ethoxylate esters, alkyl polysaccharides, block copolymers, random copolymers, trisiloxanes, CHELACTANTS™ and blends. Surfactant preference is for polyalkylene oxides, polyalkylene glycols, and alkoxylate-fatty acids. Blends are highly effective such as our organosiloxane/nonionic surfactant Dow Corning®+Pluronic® blend which use is demonstrated in our examples. Preferred commercial aqueous surfactants include Hampshire LED3A; HAMPOSYL®; TEEPOL®; TWEEN®; TRITON®; LATRON™ PLURONIC®; TETRONIC®; SURFONIC®; SYNPERONIC®; ADMOX®; DAWN®, and the like. Commercial emulsifiers for combination with organic solvent formulations include WJTCANOL®, RHODASURF®, TERGITOL® and TWEEN®. Commercial spreaders include paraffin oil. TEGOPREN®, AGRIMAX™, DOW CORNING® 211, X-77®, SILWET® and the like. Penetrants such as sodium dodecylsulfate, formamides and lower aliphatic alcohols, may be used. Alkoxylation of an active component or otherwise chemically modifying the active components by incorporating a penetrant substance is useful because formulation without additional surfactant is achieved.

Large molecules, such as compounds with maltose and polysaccharide structural components, pose problems related to cellular penetration. Addition of diatomaceous earth, carborundum, fine bentonite, clay, fine sand or alumina may be added to the compositions of the present invention to scratch the leaf surface and assist with penetration. Small quantities (0.03-0.3 percent) of sterile diatomaceous earth are preferred additions to the adjuvant formulation to enhance penetration. In some cases, such as cabbage, in which cells are tough, gentle movement of the diatoms across the leaf surface by mechanical rubbing or high pressure treatments may be employed.

In addition to the foregoing additives, the compositions of the present invention may also advantageously include one or more fertilizers. Suitable fertilizers for inclusion in the compositions, methods and systems of the present invention will be readily determinable by those skilled in the art and include conventional fertilizers containing elements such as nitrogen, phosphorus, potassium, elevated carbon dioxide, hydrogen peroxide and the like.

Nitrogenous fertilizers (i.e., fertilizers containing nitrogen) are currently preferred; particularly nitrogenous fertilizers containing ammoniacal nitrogen (that is., nitrogen in the form of ammonia or ammonium ion). Nitrate fertilizers may be included in the methods of the present invention. In particular, in cases requiring foliar fertilizers, ammonium nitrate fertilizers may be utilized. Ammoniacal fertilizers may be fed to plants at any time during or after treatment, through the root or the shoot. The amount of fertilizer added to the compositions of the present invention will depend upon the plants to be treated, and the nutrient content of the soil. Typically, the conventional fertilizer is included in an amount of between 0.1 percent and 2 percent, preferably between 0.2 percent and 1 percent, and more preferably between 0.4 percent and 0.8 percent by weight of the composition.

As noted, the compositions of the present invention may be applied to the plants using conventional application techniques. Plants nearing or at maturity may be treated at any time before and during seed development. Fruit bearing plants may be treated before and after the onset of bud or fruit formation.

The compositions of the present invention may be applied to the plant at a location including leaves, fruit, flowers, shoots, root, seed, and stem. The compositions may be applied to the leaves, seed or stem by spraying the leaves or coating the seeds with the composition. The composition may be applied to the shoot or root by spraying the shoot or root, or dusting the shoot or root, or side-dressing the root with slow-release encapsulations or formulations, or dipping the shoot or root in a bath of the composition, or drenching the soil in which the plant is being cultivated with the composition, or spray-drenching the leaves and stem of the plant such that the soil in which the plant is being cultivated becomes saturated with the composition.

Foliar application (that is., application of the composition to one or more leaves of the plant) of the compositions of the present invention is currently preferred. The composition will normally be applied to the leaves of the plant using a spray. However, other means of foliar application, such as dipping, brushing, wicking, misting, electrostatic dispersion and the like of liquids, foams, gels and other formulations may also be employed. Side dressing is also applicable. Foliar sprays can be applied to the leaves of the plant using commercially available spray systems, such as those intended for the application of foliar fertilizers, pesticides, and the like, and available from commercial vendors such as FMC Corporation, John Deere, Valmont and Spraying Systems (TEEJET®). If desired, the conjugated indole and phytocatalyst compounds may be applied to plants in rapid sequence from separate nozzles in separate reservoirs. Chemically compatible combined mixtures

may be preferred for many applications to produce improved plant growth. High foliar content of conjugated indole and phytocatalyst maintain high rates of growth during day and night, with greatest response when plants are exposed to water, nutrients, warmth and high light intensity consistent with good agricultural practices. High potency is achieved by foliar application of compositions containing one or more conjugated indoles in combination with the phytocatalyst or readily metabolized precursors, thereto.

In the embodiment wherein the root and/or shoot is dipped in a bath of the formulation, it is preferred to pulse the application of the formulation of the present invention by dipping the shoot and/or root in the bath containing the formulation for a period of time and then removing the shoot and/or root from the formulation. The dipping period may be from 10 minute to 60 minutes, and is preferably from 30 to 45 minutes.

The formulations of the present invention may also be applied to plant tissues, such as cell suspensions, callus tissue cultures, and micropropagation cultures. Such plant tissues may be treated with the formulations of the present invention by adding the formulation to the culture medium in which the plant tissues are being cultivated. For example, 50 ppm indolyl acetylglucoside may be added to an agar supported protocorm nutrient medium. Formulations may be formulated at very low concentrations without surfactant or spreader for treatments of roots and liquid suspension culture media.

In the methods of the present invention, the formulations are typically applied in the amount of between 3 gallons per acre and 100 gallons per acre, depending upon the application method. For horticulture applications, the formulations are preferably applied in the amount of between 75 gallons per acre and 100 gallons per acre. For ground-rig row crop applications, the formulations are preferably applied in the amount of between 10 gallons per acre and 40 gallons per acre. As a standard for consistent comparisons, treatments of this invention are calibrated to convential foliar spray ground rig volumes of 20 gallons per acre. For aerial applications by helicopter or airplane crop dusters, the formulations are preferably applied in the amount of between about 1 gallon per acre and about 5 gallons per acre. The formulations may be applied in a single application, or in multiple applications interrupted by periods of photosynthetic activity. Ornamentals and other tender nursery plants meant for indoor horticulture will frequently require lower concentrations and more frequent application than outdoor agricultural crops.

In general agricultural practice, withholding pesticidal application to the target crop for 2 days prior to and following treatment is recommended to prevent interference. Suitable light and temperature conditions may be achieved by treating plants at any time of day or

night. Optimal to hot temperatures, usually above 15°C and preferably above 30°C, may be required after treatment. The plants should remain exposed to the sunlight or high intensity illumination for a period of time sufficient to allow for incorporation of treatments. Usually, the plants should remain exposed to sunlight or other illumination during daylight photoperiods for at least six hours after treatments. Sufficient nutrients should be present to support healthy growth.

Throughout the growing season after treatments, either sun or artificial illumination should have an intensity and duration sufficient for prolonged high rates of photosynthesis. A minimum suitable illumination intensity is 200 μmol photosynthetically active quanta (400-700 nm) $m^{-2}s^{-1}$, with direct sunlight normally providing much higher illumination. Prior to treatment, leaf temperature should be sufficiently high for optimal growth or hotter, usually above 100°C to 35°C. After treatment, the leaf temperature will normally drop as a consequence of improved transpiration. It is preferable that the plant be exposed to at least a week of intense illumination preferably greater than 500 μmol photosynthetically active quanta $m^{-2}s^{-1}$ following application of the formulations of the present invention.

Formulations according to the present invention may be tailored for specific uses, including enhanced yield; early yield; rapid cycling through growing seasons; aftermarket; rooting; branching; flower retention; fruit optimization; using one or more conjugated indoles which have commercial impact and with which optimal growth and quality control is beneficial.

**Systems**

In addition to the methods and formulations described hereinabove, the present invention also includes a plant growth enhancing system. The system includes (a) an aqueous solution containing an amount of a phytocatalyst which provides component that supports enzymes necessary for transport of glycosides in the plant, and (b) an aqueous solution containing an amount of a glycosylator which induces indoxyl-glycosylation and (c) an aqueous solution containing an amount of indoxyl which induces growth of the plant by transport of the glycosylator to glycosidic sites in said plant. Typically, the phytocatalyst is selected from the group consisting of ammoniacal nitrogen, soluble manganese, and soluble iron and combinations thereof, although any of the phytocatalyst components described hereinabove may be employed in the systems of the present invention. The glycosylator employed in the systems of the present invention may also be selected from those described above. Preferred glycosylators for use in the systems of the present invention include, but are not limited to, alcohols. organic acids, bicarbonates and alkyl

glycosides and combinations thereof. One preferred system according to the present invention includes a formulation of urea-formaldehyde, ammonium sulfate, MnEDTA and FeHEDTA as the phytocatalyst; with elevated carbon dioxide and potassium formate as the glycosylator; and indoxyl as the PGR.

The following examples are provided to further illustrate the present invention, and should not be construed as limiting thereof. In these examples, manganese EDTA, ferric EDTA, ferric HEDTA, Dow Corning® surfactants (CWN), and purified water were obtained from Dow Chemical Company. Ethanol (Ethan), ammonium sulfate (AMS), ammonium nitrate (AMN), phosphate (KP), and conjugated indoles were obtained from Fisher Scientific. CPGRs, ethylenediamine tetraacetic acid (EDTA) and synthetic components of conjugated indoles were obtained from Sigma. Pluronic® surfactants (AKN) were obtained from BASF.

In these examples, "1" means liter; "ml" means milliliter; "cm" means centimeter; "cm$^2$" means centimeters squared; "μg" means micrograms; "gm' means grams; "mM" means millimolar; "ppm" means parts per million based on weight; and "percent"or "percent" means percent by weight (of the composition).

Following are examples of specific formulation according to the present invention, which may advantageously be employed in the methods of the present invention to treat plants and to enhance growth in plants to increase transport of glycosides in plants. The following exemplary formulations are intended to provide further guidance to those skilled in the art, and do not represent an exhaustive listing of formulations within the scope of the present invention.

**First Exemplary Formulations: Foliar**

| Composition | Preferred concentration | Broad Range Concentration |
|---|---|---|
| Indoxyl glycoside | 1mM | 1mM to 6mM |
| Isatin | 1mM | lmM to 3mM |
| Isatoxime | 3mM | 1mM to 3mM |

Using any of the above composition, mix the preferred amount in water. Spray on foliage at a volume of 20 gallons per acre. Allow approximately a week or more between treatments.

**Second Exemplary Composition: Root Immersion**

| Composition | Concentration |
|---|---|
| Indoxyl glucuronide | 1-3mM |
| AMS | 50mM |
| Manganese | 20ppm |
| Iron | 20ppm |

The cyclohexylammonium salt of indoxyl β-D-glucoronide was purchased from Sigma and formulated with the ammonium sulfate, manganese and iron. A solution of the formulation was applied to radish foliage with 1 gm/liter CWN/AKN surfactant blend. When compared to an identical control formulation without the indoxyl glucuronide, the above formulation provided a root increase of equal to or greater than 30 percent.

**Third Exemplary Composition: Foliar**

| Composition | Concentration |
|---|---|
| Indoxyl glucoside | 1-4mM |
| AMS | 50mM |
| Manganese | 20ppm |
| Iron | 20ppm |

Indoxyl β-D-glucoside was purchased from Sigma and formulated with the ammonium sulfate, manganese and iron. A solution of the formulation was applied to radish foliage with 1 gm/liter CWN/AKN. The surfactant was presolubilized in equal parts of Ethan and then diluted. When compared to an identical control formulation without the indoxyl glucuronide, the above formulation provided a root increase of equal to or greater than 30percent. For indoxyl glucoside, the proper dose for radish is between 25 to 75 μg per plant and preferably between 40 to 50 μg per plant. For example, this dose may be achieved by applying 1.2 to 1.5 ml/1000 $cm^2$ leaf surface area of 2 mM concentration (1.05 gm/liter) of indoxyl glucoside, trihydrate. This is the equivalent to an application of 10 to 20 gallons per acreat at a preferred volume of 15 gallons per acre of 3 mM concentration (1.05 gm/liter) of indoxyl glucoside, trihydrate.

The proper dose of indoxyl glucoside for Canola is between about 10 to 75 μg per plant and preferably between about 20 to 30 μg per plant. For example, this dose may be achieved by applying 1.2 to 1.5 ml/1000 $cm^2$ leaf surface area of 1 mM concentration (0.335 gm/liter) of indoxyl glucoside, trihydrate.

**Fourth Exemplary Composition: Root Immersion**

| Composition | Concentration |
|---|---|
| Indoxyl glucoside | 1gm/liter |
| AKN surfactant | 2gm/liter |

Provide fertizers to crop so that nutrient supplementation is optimized for desired yield. Dissolve contents in 1 liter of water. Thoroughly mix the solution until clear. Upon expansion of first true leaves, apply the solution to the foliage of the crop at a volume of 20 gallons per acre. In instances where nutrients are not optimized for a given crop, supplement the exemplary formulations with the following minimal plant nutrients:

| | |
|---|---|
| AMS | 7gm |
| KP | 1gm (adjust to pH 7 to pH 8) |
| MnEDTA (12 percent Mn) | 0.2gm |
| FeEDTA (13percent Fe) | 0.2gm |

Generally, the activity of indican (indoxyl glycoside) is supported by the presence of iron, manganese and ammonia. For consistent field activity, 100 mM ammoniacal nitrogen, 20 ppm Mn and 15 ppm Fe are the minimal available nutrients required for enhancement of plant growth by exposure to indican.

**Fifth Exemplary Composition: Foliar**

| Composition | Concentration | Percentage |
|---|---|---|
| 3 mM indoxyl glucoside | 1 .05gm/liter | 10 percent |
| 50 mM AMN | 6.6gm/liter | 70 percent |
| 3.5 mM KP dibasic | 0.61gm/liter | 5 percent |
| 28 ppm Mn (dry Mn-EDTA) | 0.2gm/liter | 2.5 percent |
| 24 ppm Fe (dry Fe-EDTA) | 0.2gm/liter | 2.5 percent |
| CWN/AKN surfactants | 1.1gm/liter | 10 percent |
| TOTAL | 9.76gm/liter | |

Apply to foliage at 20 gallons per acre.

Although specific features of the invention are described with respect to one example and not others, this is for convenience only as some feature of one described example may be combined with one or more of the other examples in accordance with the methods and formulations of the invention.

Other permutations of the methods and formulations of the invention will occur to those skilled in the art and are within the following claims:

What is claimed is:

1. A method for treating plants and for enhancing plant growth, comprising the step of

applying an effective amount of one or more compounds selected from a group consisting of conjugated indoles, salts and derivatives of said conjugated indoles and combinations thereof, to said plant.

2. The method of claim 1, wherein one or more of said compounds comprises indoxyl glycoside.

3. The method of claim 2, wherein an effective amount comprises indoxyl glucoside in an amount between about 1 mM to 6 mM concentration.

4. The method of claim 1, wherein one or more of said compounds comprises indoxyl mannoside.

5. The method of claim 1, wherein one or more of said compounds is selected from a group consisting of, indoxyl glucoside, indoxyl glucuronide, indoxyl mannoside, isatin, isatan, isatoxime, indirubin, indole carboxylate, indole $(acyl)_n$glycoside, and isomers and salts thereof.

6.The method of claim 5, wherein said indole $(acyl)_n$glycoside comprises one or more indoxyl $(acetyl)_n$glycosides, wherein n=1-5.

7. The method of claim 1, further comprising the step of applying one or more phytocatalysts.

8. The method of claim 7, wherein one or more of said phytocatalysts comprises,

ammonium at 100 millimolar concentration ∓ 20 percent;

manganese at 30 parts per million concentration ∓ 20 percent: and

iron at 15 parts per million concentration ∓ 50 percent.

9. The method of claim 7, wherein one or more of said phytocatalysts comprises one or more nutrients selected from a group consisting of iron, manganese and nitrogen.

10. The method of claim 9, wherein said nitrogen nutrient, comprises ammoniacal nitrogen.

11. The method of claim 1, wherein said effective amount is between about 1 ppm to 2,500 ppm.

12.A formulation for treating plants and for enhancing plant growth, comprising, one or more compounds selected from a group consisting of indoxyl glycosides, salts and derivatives of said indoxyl glycosides and combinations thereof.

13. The formulation of claim 12, wherein one or more of said compounds comprises indoxyl glucoside.

14. The formulation of claim 12, wherein one or more of said compounds comprises indoxyl glycoside between about 1 mM to 6 mM concentration.

15. The formulation of claim 12, wherein one or more of said compounds comprises indoxyl mannoside.

16. The formulation of claim 12, wherein one or more of said compounds is selected from a group consisting of. indoxyl glucoside, indoxyl glucuronide, indoxyl mannoside, isatin, isatan, isatoxime, indirubin, indole carboxylate, indole $(acyl)_n$glycoside, and isomers and salts thereof.

17. The formulation of claim 16, wherein said indole $(acyl)_n$glycoside comprises one or more indoxyl $(acetyl)_n$glycosides, wherein n= 1-5.

18. The formulation of claim 13, further comprising the step of applying one or more phytocatalysts.

19. The formulation of claim 18, wherein one or more of said phytocatalysts comprises one or more nutrients selected from a group consisting of iron, manganese and nitrogen.

20. The formulation of claim 19, wherein one or more of said phytocatalysts comprises.

ammonium at 100 millimolar concentration ∓ 20 percent;

manganese at 30 parts per million concentration ∓ 20 percent; and

iron at 15 parts per million concentration ∓ 50 percent.

21. The formulation of claim 19, wherein said nitrogen nutrient comprises ammoniacal nitrogen.

22. The formulation of claim 14, further comprising one or more surfactants.

23. A formulation for treating plants and for enhancing plant growth, comprising, one or more indoxyl glycosides in an amount between about 1 mM to 6 mM concentration; and

one or more phytocatalysts comprising, iron, manganese and nitrogen.

24. The formulation of claim 23, wherein one or more of said phytocatalysts comprises,

ammonium at 100 millimolar concentration ∓ 20 percent;

manganese at 30 parts per million concentration ∓ 20 percent; and

iron at 15 parts per million concentration ∓50 percent.

25. The formulation of claim 24, further comprising one or more surfactants.

26. A method for treating plants and enhancing plant growth, comprising the step of, applying one or more compounds selected from a group consisting of cyclic alkyl glycosides; salts and derivatives of said cyclic alkyl glycosides; cyclic acyl glycosides; salts and derivatives of said cyclic acyl glycosides; and combinations thereof, to the plants.

27. The method of claim 26, wherein one or more of said compounds comprises one or more indoxyl glycosides; salts and derivatives of said indoxyl glycosides and combinations thereof.

28. A formulation for treating plants and enhancing plant growth, comprising, one or more compounds selected from a group consisting of cyclic alkyl glycosides; salts and derivatives of said cyclic alkyl glycosides; cyclic acyl glycosides; salts and derivatives of said cyclic acyl glycosides; and combinations thereof.

29. The formulation of claim 28, wherein one or more of said compounds comprises one or more indoxyl glycosides; salts and derivatives of said indoxyl glycosides and combinations thereof.

# INTERNATIONAL SEARCH REPORT

Intern: al Application No
PCT/US 01/02081

**A. CLASSIFICATION OF SUBJECT MATTER**
IPC 7 A01N43/38

According to International Patent Classification (IPC) or to both national classification and IPC

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, BIOSIS, WPI Data, PAJ, CHEM ABS Data

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

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[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

| Date of the actual completion of the international search | Date of mailing of the international search report |
|---|---|
| 19 April 2001 | 02/05/2001 |

| Name and mailing address of the ISA | Authorized officer |
|---|---|
| European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31–70) 340–2040, Tx. 31 651 epo nl, Fax: (+31–70) 340–3016 | Bertrand, F |

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| Category ° | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
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